Title: Potassium Fertilization and Its Impact on Yield, Quality, and Winter Hardiness of Alfalfa

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Abstract: (Limit 200-300 words)
There are multiple factors that determine the appropriate rate of K fertilization in alfalfa, one of them is soil clay mineralogy. Potassium becomes immobile when the soil smectite-to-illite clay mineralogy ratio is greater than 3.5. The goal of this study was to determine the effect of K rate and application timing, varietal fall dormancy, and harvest stress on forage yield, nutritive value, and winter survival in soils with different clay mineralogy. Experiments were established in Lisbon and Milnor, ND in 2019. Milnor and Lisbon have soils with with a smectite-to-illite ratio >3.5 and <3.5, respectively. The experimental design was a randomized complete design with a split-plot arrangement and four replicates, where the varieties Presteez RR, Stratica RR, and L-450 RR were assigned to the main plots. The subplots were a factorial combination of K rates (0, 150, and 300 lbs K_2O/acre) and two application timings. Two harvest timings were also included (stressed and non-stressed treatments). Higher K fertilizer rates did not increase alfalfa forage yield. Increase of K application did result in a decrease in nutritive value (TDN). Alfalfa stand counts decreased from fall to spring. Alfalfa harvested on 18 September (stress treatment) had a stand reduction from fall to spring between 47.1 and 51.4% and alfalfa harvested on 16 October (non-stress treatment) had a stand reduction of 16.5 to 26.9%.The differences in soil available K in the fall between locations supports that the higher smectite-to-illite clay ratios soil may have lower availability of K to plants. Summing soil available K in the fall and K applied as fertilizer, the K accumulated in the plant explains less than 25% and 54% of the K available at the highest fertilization rate at the <3.5-clay ratio soil and >3.5-clay ratio soil, respectively. Root protein content was lower when harvested in September.

Introduction:

Alfalfa (*Medicago sativa* L.) is a cool-season forage crop that provides an excellent feed for livestock. The relative forage value of alfalfa is higher than any other forage crop (Perić and Srebric, 2016). Alfalfa forage yield depends on stand establishment, proper harvest times, and fertilization. Potassium is often under applied due to the high cost of fertilizers containing potassium (Lloveras et al., 2012). However, inadequate potassium levels in the soil can contribute to winterkill of alfalfa plants (Hawkesford et al., 2011; Jungers et al., 2019a). In the Upper-

Midwest, growers are hesitant to harvest alfalfa in the fall, due to concern of winterkill. Fall harvest, though, can increase total seasonal forage yield without depleting persistence or nutritive value (Berti et al., 2012).

There is still a lack of understanding on how management and the environment affects alfalfa winter survival, including potassium levels (Berg et al., 2018). There is knowledge that potassium has an important role in alfalfa growth, however, the effects of K fertilization of alfalfa is inconsistent (Jungers et al., 2019). Previous studies support that soil fertility of P and K nutrients positively impact alfalfa persistence, forage yield, and winter survival, however, the impact of P fertilization has been evaluated more exhaustively than K fertilizers (Berg et al., 2018). Potassium fertilization rate depends on the current K levels in the soil, the alfalfa tonnage removed from the previous year, and the soil's clay chemistry (Franzen and Berti, 2017).

A previous study examined how potassium fertilization rates need to be adjusted in certain soil clay mineralogies (Breker et al., 2019). This study found that soils with a smectite-to-illite ratio greater than 3.5 require higher amounts of K, due to the clay types tying up the available K. This recent finding emphasizes the importance of better understanding K fertility and adjusting recommended rates in certain environments. Fertilization of K has shown to have a positive effect on forage yield when soil test K levels are low (Jungers et al., 2019a), however, increased K application does not always result in higher yield (Berg et al., 2018). Higher rates of K have shown to reduce forage nutritive value and increase forage K concentration.

Potassium has an important role in numerous physiological processes within a plant, but the process vital for winter survival is nutrient storage prior to dormancy (Lu et al., 2018). This is due to K playing a strong role in transportation of ATP, protein, starch, and other nutrients, to the roots. Previous findings indicated that protein reserves are important for tolerance of both defoliation and winter stresses (Volenec et al., 1996). Analyzing taproots for total protein and starch content has potential to understand potassium's role in that nutrient storage. Prior analyses on sugar reserves within taproots have found insignificant content difference among potassium rates (Berg et al., 2018)

This study focuses on the effects K fertilization has on alfalfa yield, nutritive value, and persistence of differing fall dormancies with variable rate and harvest times. It evaluates how protein and starch reserves within alfalfa roots differ among K rates and application timing. Lastly, this study looks at the interaction between fall dormancy and protein and starch reserves among K fertilization.

Materials and Methods:

Two separate sites were established for this study. One location is East of Lisbon, ND (46°26'N, -97°11'W, 325 m elevation). The second location is in Milnor, ND (46°16'N, -97°28'W, 337 m elevation). Soil type in Lisbon is Ulen fine sandy loam soil and the soil type in Milnor is Hecla-Garborg fine sandy loam soil, (Ulen: sandy, mixed, frigid Aeric Calciaquoll; Hecla: sandy, mixed, frigid Oxyaquic Hapludoll; Garborg: sandy, mixed, frigid Typic Endoaquoll) (Web Soil Survey, 2009).

The two locations differ in their smectite-to-illite ratio of clay mineralogy. Milnor has soil that immobilizes potassium, having a smectite-to-illite ratio greater than 3.5 and Lisbon has soil that allows potassium to be more mobile having a smectite-to-illite ratio less than 3.5.

The previous crops for Lisbon and Milnor were soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays*) respectively. The Milnor site had a broadcasted cereal rye (*Secale cereale* L.). For the Milnor site, mowing and raking was required in order to remove the corn stalk residue and allow for the disks on the planter to properly sow. Both research plots are located in previously-established no-till sites. No-tillage is continued as the management strategy for both fields.

Three alfalfa varieties Presteez RR, Stratica RR, and L-450 RR were used. All of the varieties are glyphosate-tolerant alfalfa. Each of the varieties had a different fall dormancy score.

Table 1. Varietal characteristics and the seeding rates for planting at both Milnor and Lisbon sites in 2019

Variety	FD†	WS§	Germination	Purity	PLS¶	PLS planted
			%	%	%	kg ha^{-1}
RR Presteez	3.2	1.2	88.0	66.0		19.31
Stratica	4.3	2.0	88.0	66.0		19.31
L450 RR	5.0	1.4	80.0	65.98	52.8	21.22

†FD: fall dormancy. §WS: winter survival. ¶PLS: pure live seed.

The seeding rate for all three alfalfa varieties was 11.23 kg of pure live seed/ha (10 lbs/acre) of alfalfa. The rate of inoculant was 0.20 g/plot of Pre Vail inoculant (*Sinorhizobium meliloti, Rhizobium leguminosarum biovar trifolii, Azospirillum brasilense* - growth promoter).

The experiment was a randomized complete block design with a split-plot arrangement and four replicates. The main plot was the variety and the subplots a factorial combination of K rates and application timing. In addition, in the fall of the seeding year, each plot was split in half, with one-half of the plot harvested mid-September, stressing the alfalfa, and the second-half of the plot harvested before first frost on 16 October, non-stressing the alfalfa (Table 2).

Table 2. Potassium treatments and the timing of application (split application at seeding or ½ at seeding and ½ in the fall) at two location sites in 2019.

Rate K_2O		Date applied			
kg ha^{-1}(lbs/acre)		Lisbon		Milnor	
0	(0)				
168	(150)	13 May		15 May	
168,	(150) S	30 July	19 Sept	29 July	19 Sept
336	(300)	13 May		15 May	
336	(300) S	30 July	19 Sept	29 July	19 Sept

Plastic pipes (10.2-cm) were inserted in each subplot to mark 0.19 m^2 area denoted for measuring plant density. These pipes indicate where the stand count sample area and allows the sampling of biomass within a 0.19 m^2 area.

Prior to planting, soil cores were collected for each of the four blocks at 0-15-cm and 15-61-cm to give a baseline of current soil conditions. The baseline soil sampling was sent to the soil testing laboratory to measure NO_3-N, P, K, pH, and organic matter (OM). The following methods were used: NO_3-N, colorimetric determination by trans-nitration of salicylic acid method (Vendrell and Zupancic, 1990); P, Olsen procedure using Brinkmann PC 910 colorimeter; K, ammonium acetate method using Buck Scientific Model 210 VGP atomic absorption spectrophotometer; pH, calcium chloride method; OM, loss of ignition.

Table 3. Soil sampling test results of 0-15-cm cores taken at two locations in 2019

Location	NO_3-N	P	pH	OM	Date	K level among treatments				
						1	2	3	4	5
	kg ha^{-1}	ppm		%		----------------ppm---------------				
Milnor	8.96	6.00	7.28	2.75	15 May	91.25	96.5	100	90.2	86.9
					8 Oct.	74.2	81.6	90.2	90.8	123
Lisbon	8.68	24.3	5.70	2.47	10 May	76.3	78.1	78.8	80.1	78.9
					9 Oct.	63.9	87.5	86.1	121	128

Fertilization of MAP 11-52-0 was applied in Milnor in order to increase P and ensure all other nutrients were non-limiting. Beet lime was also applied in accordance to the rate of 4.48 Mg ha^{-1} to raise pH by 0.5. For weed control, glyphosate [N-(phosphonomethyl) glycine] was applied at 1.1 kg acid equivalent (a.e.) ha^{-1} as needed. Insecticide (Lambda-cyalothrin) was applied at a rate of 0.09 kg a.e. ha^{-1} to control potato leaf hoppers (*Empoasca fabae Harris*) at both locations, two times in the growing season.

Table 4. Rates and timings of fertilizers and pesticides applied at two locations in 2019.

Location	Glyphosate a.e. †		P_2O_5 (11-52-0)		Beet lime		Insecticide (Lambda-cyalothrin) a.e.	
	kg ha^{-1}	date	kg ha^{-1}	date	Mg ha^{-1}	date	kg ha^{-1}	date
Lisbon	1.1	29 May, 17 Ju.			4.48	18 Jun	0.09	17 Jul, 14 Aug
Milnor	1.1	29 May, 17 Jul	100	3 Jun			0.09	17 Jul, 14 Aug

†a.e.: acid equivalent

Field Establishment, Year 1

In May of 2019, the alfalfa was planted and established at the two locations. The alfalfa was seeded at a depth of 0.95-cm with an 8-row continuous plot drill XL (Wintersteiger, Salt Lake City, UT). Experimental units are 6.1-m long with the eight rows spaced apart 15.2-cm.

Alfalfa was harvested twice, targeting the first cutting to late bud to 10% bloom. At harvest, height, stage, and a stand count of the pre-marked 0.6 m^2 was noted. Plant height was measured before each harvest from each sub-plot. Plant height was measured with a measuring stick from the ground level to top leaf. Plants growing stage was recorded during each harvest. Stand counts were taken within the 0.19 m^2 area denoted by the pipes. The alfalfa was harvested using a six-row flail forage harvester (Carter, Brookston, IN). At first harvest, each full plot was harvested together. Whole plot fresh weight was recorded, and a fresh sample was taken and weighed.

The harvester cut the alfalfa to down to 7.6-cm stubble. Using hand sickles, all missed plants were cut down to 7.6 cm stubble to improve uniformity. Harvested samples of wet herbage were dried at 37.8°C for 72 h to determine dry matter yields.

Table 5. Planting and harvest dates at two locations.

Location	Seeding	First cut	Second cut (stressed)	Second cut (non-stressed)
Lisbon 2019	10 May	30 Jul.	19 Sept.	16 Oct.
Milnor 2019	15 May	29 Jul.	19 Sept.	16 Oct.

At the second and final cut of the year, plots were separated by “a” and “b” halves. The area harvested per half of a plot is 4.6 m^2. Half of the plot was harvested on 18 September to stress the alfalfa, assuming there were sufficient temperature and moisture for regrowth, drawing energy from root reserves.

The non-stressed second cut occurred in October, before the first hard frost. At this harvest time, root samples were collected for measuring taproot starch and protein reserves. Root samples were dug out at a depth of 15.2-cms. Samples were cut above the crown, washed, dried, and then stored in a freezer at -80°C.

Forage Quality Analysis

Aboveground alfalfa samples were ground to a 1-mm size. The ground samples were analyzed with a near-infrared spectroscopy, NIR (XDS Analyzer, Foss-Sweden Model 6500, Minneapolis, MN, USA)
This will analyze the feed quality of each alfalfa sample: crude protein (CP), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber digestible (NDFD), in vitro dry matter digestibility (IVDMD). The NIR was calibrated for measuring pure alfalfa. The nutritional potassium value was also analyzed to determine any additional effects of the treatments. Analyses follow the methodology of Abrams et al. (Abrams et al., 1997).

Root Reserve Analysis

The stored root samples were analyzed for starch and protein reserve content. Root samples were separated into 50-mL flasks and covered with a Kimwipe. All samples were freeze-dried using the Virtis SP Scientific Sentry 2.0 at the USDA-ARS, Edward T. Schafer Agricultural Research Center in Fargo. Freeze-drying was at -70°C and 200-mTorr for 48-h. All samples were ground and passed through 1-mm screen and separated into two, 5-mL tubes. Samples were then stored in -80°C freezer until analysis of protein and starch.

Starch content was determined by the method of Smith and Zeeman (2006). For analyzing starch, 30-mg of root sample was placed in 100-µL of 0.2-unit Na acetate buffer (pH 5.1). The starch was digested by adding 0.2 unit of amylo glucosidase and 40 units of α-amylase. The tubes were incubated at 55°C. The samples was then microcentrifuge at 14,000 × *gn*. Within the supernatant, glucose were determined using glucose oxidase. The concentration of starch was estimated as 0.9 times the glucose concentration. Starch analysis is underway; the method has been calibrated, but starch analysis has not been completed yet.

Protein content was determined by the method of Bradford (1976). Analyzing N reserves requires 30-mg of root sample to be suspended in 1 mL of 100-mM sodium phosphate buffer (pH 6.8). One part of dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted using four parts of distilled water. A volume of 5-mL of diluted dye reagent was added to 50-µL of the root extract. The solution was vortexed and then incubated in the dark for five minutes. The absorbance of the mixture was measured at 595-nm against a blank (50-µL distilled water) by using an ultraviolet-visible (UV-VIS) Genesys spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Statistical Analysis

The data collected was analyzed using the procedure MIXED procedure of SAS 9.4 (SAS Systems Inc., Cary, NC) with the repeated measures function to analyze harvest timings (cuts). Location, variety, treatments and cuts were all considered fixed effects for the analysis. Location was analyzed as fixed effect since we were comparing the two sites differential clay ratio composition. For means separation treatment an LSD at 95% confidence of interval was used.

Project Objectives and Corresponding Results:

PROJECT OBJECTIVES	**PROJECT RESULTS**
To determine the effect of K fertilization on alfalfa yield, quality, and persistence in alfalfa of different fall dormancy and with variable rate application and harvest stress.	1) ***Alfalfa forage yield:*** No significant effect of K fertilization or variety was observed on total forage yield in the seeding year. 2) ***Alfalfa forage nutritive value*****:** Potassium fertilization or variety did not affect crude protein concentration or fiber components (NDF, ADF, ADL). Ash content was higher and total digestible nutrients (TDN) were significantly lower with higher rates of K. TDN was highest in the first cut across all varieties. 3) ***Alfalfa persistence:*** stand counts decreased from fall to spring in all treatments. Alfalfa harvested on 18 September (stress treatment) stand reduction ranged between 47.1 and 51.4%. Alfalfa harvested on 16 October (non-stress treatment) had significantly lower plant stand reduction ranging between 16.5 and 26.9%. Potassium fertilization or varieties did not have an influence on stand reduction. 4) ***Potassium fate:*** Potassium fertilizer applied to the soil with smectite-to-illite ratio >3.5 did not increase available K soil level between the spring and fall. Potassium extracted by alfalfa biomass could not explain the fate of K. Potassium unaccounted for in the high smectite-to-illite ratio soil ranged between 100-300 lbs/acre, indicating it is likely the K is being fixed by soil clays.
To determine the changes in root carbohydrate reserves in alfalfa with different K rates and application timing.	1) Root protein concentration was not influenced by K rate or K application timings. 2) Root starch concentration analysis has not been completed at the closing of this report.
To determine the interaction between fall dormancy and K fertilization on root storage of protein and starch.	1) No interaction was observed between fall dormancy and K fertilization on root storage of protein on the seeding year of alfalfa.

Results and Discussion:

Forage yield and nutritive value:

In the 2019 seeding year, K fertilization rates and timings did not affect alfalfa seasonal forage yield ($P \geq 0.05$) (Table 6). The total dry matter forage yield ranged between 7.0 and 7.4 Mg ha^{-1} (3.1-3.3 tons/acre). There was also no significant interaction between treatments, cuts, nor varietal differences. Potassium fertilization did not affect ADL, NDF, CP, and NDFD. There was, however, an effect of K treatments on total ash and TDN concentration. With increased K application, there was an increase in ash content and a decrease in TDN (Table 6). Alfalfa with no potassium fertilization had the lowest ash concentration and highest TDN in all cuts. The highest ash concentration was observed for the 336 kg ha^{-1} (300 lbs/acre) rate applied at seeding. TDN was affected by the interaction between variety and cut. The variety with FD3 (Presteez) had the highest TDN across all three cuts (Table 7).

Table 6. Alfalfa seasonal forage yield, and TDN and ash concentration affected by K fertilization, application timing and harvest time averaged across two locations, Lisbon and Milnor, ND.

K rate	Forage yield	Forage yield	TDN	Ash concentration (%)		
(lbs/acre)	(Mg ha^{-1})	(tons/acre)	(%)	Cut 1	Cut 2-18 Sep	Cut 2- 16 Oct
0	7.0	3.12	61.0	7.5	7.8	8.4
150-seeding	7.3	3.26	59.8	7.9	8.1	8.6
150-split	7.0	3.12	60.5	7.6	8.1	8.5
300-seeding	7.4	3.30	59.4	8.1	8.3	8.7
150-split	7.2	3.21	59.7	7.5	8.2	8.6
LSD	NS	NS	0.8		0.1	

Table 7. Total digestible nutrients (% of dry matter) affected by variety and harvest date (cut) averaged across two location, Lisbon and Milnor.

Variety	Cut 1	Cut 2-18 Sep	Cut 2-16 Oct
Presteez	65	59	58
Stratica	64	58	57
LR4500	64	58	57
LSD (Var x cut)		3	

Alfalfa stand persistence

Plant stands in the fall ranged between 140 and 151 plants m^{-2} in the alfalfa harvested on 18 September and ranged between 97 and 113 plants m^{-2} in the alfalfa harvested on 16 October. Potassium fertilization or application timing did not have an effect on fall stands. In the spring of 2020, plant density ranged between 65 and 68 plants m^{-2} for the stressed treatment and 71-79 plants m^{-2} for the non-stressed treatment.

Alfalfa stand counts decreased from fall to spring in all treatments (Table 8). Alfalfa harvested on 18 September (stress treatment) had a stand reduction between 47.1 and 51.4%. Alfalfa harvested on 16 October (non-stress treatment) had lower stand reduction than the stressed treatment ranging between 16.5 and 26.9%. Potassium fertilization or varieties did not have an influence on stand reduction. In the stressed alfalfa, K fertilization, application timings or varieties did not affect plant stand reduction, but there was a significant effect of location. Stand reduction in alfalfa across all treatments in Lisbon (58.8%) was much higher than in Milnor (38.8%). This could be explained by the lower pH and the sandy texture of Lisbon soil along with the extreme low soil potassium. Alfalfa plants were likely more stressed going into the winter in Lisbon than in Milnor explaining the overall much higher stand reduction.

The higher stand reduction in the stressed treatment was expected. It is not recommended to harvest alfalfa in the month of September in North Dakota, because at this time the plant will produce new shoots using the root reserves, increasing its susceptibility to winter kill. In September until mid-October there are still enough temperature and moisture for the plant to grow new shoots, but not enough time to replenish the root reserves before the hard frost. We wanted to see if K fertilization could overcome the stress caused by harvesting too early in the fall. At least in the seeding year, we did not see a clear effect.

Table 8. Alfalfa plant density affected by K fertilization, application timing, and time of fall harvest averaged across three varieties and two locations, Milnor and Lisbon, ND.

K rate (lbs/acre)	Fall stand 9-18	Fall stand 10-16	Spring stand		Stand reduction from fall to spring	
	Stressed	Non-stressed	Stressed	Non-stressed	Stressed	Non-stressed
	----------------------------Plants m^{-2}			----------------------------	--------% reduction--------	
0	151	100	65	71	51.4	25.6
150-seeding	145	102	68	74	49.4	25.4
150-split	142	106	70	73	47.1	26.9
300-seeding	140	97	68	79	47.7	16.5
150-split	141	113	68	78	47.2	27.8
P<F	NS	NS	NS	NS	NS	NS
Location						
Milnor	121	113	70	75	38.8	17.4
Lisbon	166	94	66	74	58.8	31.6
P<F			NS	NS	0.04	NS

Soil K and K accumulated in alfalfa biomass

The interactions among soil K and K accumulation across locations, treatments, and harvest times are indicated in Table 9.

At the Milnor site, potassium levels ranged from 87.0 to 100.4 ppm in May and from 74.2 to 122.6 ppm in October. The treatments with no K fertilizer or 168 kg ha^{-1} (150 lbs/acre) of K_2O at seeding had significantly lower soil K levels in the fall than treatments with higher fertilizer rates (Table 10). Soil test levels were lower in the fall for the check without fertilizer application or the168 kg K_2O ha^{-1} treatment and remained the same for the treatments with 168 kg ha^{-1} split application and the 336 kg ha^{-1} rate applied at seeding. The soil K level increased between the spring and fall testing only for the split-applied highest rate of K.

In the Lisbon site, soil K levels were significantly higher in the fall with the fertilization of 336 kg K_2O ha^{-1} at seeding or split-application. This suggest that K stays available in the soil for longer than in Milnor soil. However, this could also have been due to higher K uptake by alfalfa since in Milnor alfalfa yield was much higher than in Lisbon. For Milnor, the highest soil K averaged across sampling times was in the treatment with 336 kg ha^{-1} of K_2O applied at seeding, while in Lisbon the highest soil K was with the 336 kg ha^{-1} of K_2O split-application (Table 10). For aboveground biomass K accumulation, both Milnor and Lisbon had significantly lower levels in October than harvested samples in the first cut, which was an interaction across all treatments (Table 11). Potassium luxury consumption was not observed in Milnor site except for the 336 kg ha^{-1} of K_2O applied at seeding and it was not observed in the Lisbon site for the two highest fertilizer treatments. We believe a combination of acid soil pH, extremely low K soil levels, and a sandy texture, limited plant growth preventing the plant from using the available K on the fertilized treatments.

Table 9. Analysis of variance of soil K and K accumulated in alfalfa biomass for two locations (Loc), five fertility treatments (Trt) and two sampling times (stimes) in 2019 in Lisbon and Milnor, ND

	Soil K			K accumulated		
Source	df	Mean square	Pr > F	df	Mean square	Pr > F
Loc	1	1302	0.0535	1	73028	<.0001
Trt	4	5143	<.0001	4	1105	<.0001
Loc x trt	4	1054	0.0178	4	281	0.0200
Stime	1	4602	0.0003	1	59775	<.0001
Loc x stime	1	5930	<.0001	1	9830	<.0001
Trt x stime	4	6152	<.0001	4	495	0.0005
Loc x trt x stime	4	522	0.1994	4	106	0.3430
Rep	3	11590	<.0001	3	27	0.8333
Residual	217	345		216	94	

Table 10. Soil K variation with five fertilizer treatments and two sampling dates at two locations in 2019

Location	Sampling date	Harvest date	0	168	168S	336	336S
			------------------------- soil K level (ppm)--------------------				
Milnor	15 May	29 Jul.	91.2a†	96.5a	100.4a	90.2a	87.0b
	8 Oct.	16 Oct.	74.2b	81.6b	90.2a	90.8a	122.6a
Mean			82.7B§	89.0B	95.3A	90.5B	103.3A
Lisbon	10 May	30 Jul.	76.3a	78.1a	78.8a	80.0b	78.9b
	9 Oct.	16 Oct.	63.9a	87.5a	86.0a	120.0a	127.8a
Mean			70.1C	82.8B	82.5B	100.3A	103.0A

†Small case letters compare means between two sampling/harvest dates within same treatment and same location.

§Uppercase letter compare means among treatments averaged across sampling/harvest dates within a same location. For treatments, 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first cut, 84 kg K_2O ha^{-1} applied in mid-Sept; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first cut, 168 kg K_2O ha^{-1} applied in mid-Sept.

Available K unaccounted for

We estimated how much potassium was unaccounted for in treatments with fertilizer application. For this, the soil K available in the spring plus the K applied as fertilizer were summed up. Then the K taken up by the alfalfa biomass in both cuts and the soil K available in the fall were summed up as well. The difference between both was what we called it available K unaccounted for. Possible fates of available K we did not measure were available K below 15-cm in depth in the spring sampling, K from soil or fertilizer leached below 15-cm soil depth by the time of fall sampling, K in the alfalfa root tissue, and Kfixed by soil clay particles. Thus, we assumed the fate of the K we indicate as unaccounted for, likely followed one of these possible fates. We did take samples at 120-cm depth in the spring of 2020 to account for possible leached K. In addition, we are analyzing soil samples for non-exchangeable K to determine if some of the K in the fertilizer was adsorbed by the clays. For this, soils samples of the highest fertilizer rates and the check are being analyzed.

The analysis of variance indicated significant differences between locations, treatments, and the interaction between locations, varieties, and treatments for unaccounted-for-available K (Table 12). For all varieties and at both locations, as fertilizer rate increased unaccounted-for-K increased as expected. Milnor soil had less Kthat was not accounted for, mainly because forage yield was higher, so was the K extraction and removal from the field with the biomass. However, higher forage yield only explains in part the differences between Milnor and Lisbon soils. Milnor and Lisbon soils had 279 and 320 kg ha^{-1} of unaccounted available K, respectively, at the highest fertilization rate. The K accumulated in the alfalfa aboveground biomass explains about 54% and 25% of the K unaccounted for in Milnor and Lisbon, respectively, at the highest fertilizer rate.

The K unaccounted for could have been lost by leaching below 15-cm depth or could have been fixed within the exchange sites of soil's clay colloids or between clay layers, which has been found more common within soils with a >3.5 clay ratio soils (Breker et al., 2019).

Table 11. Aboveground biomass K accumulation variation with five fertilizer treatments and two harvest dates at two locations in 2019

Location	Sampling date	Harvest date	0	168	168S	336	336S
			----------Biomass K accumulation level (kg ha^{-1})----------				
Milnor	15 May	29 Jul.	89.6a†	104.5a	92.7a	108.6a	91.2a
	8 Oct.	16 Oct.	48.8b	46.2b	47.3b	63.1b	58.8b
Sum			138.4B§	150.7B	140.0B	171.7A	150.0B
Lisbon	10 May	30 Jul.	45.4a	56.6a	44.5a	53.1a	48.0a
	9 Oct.	16 Oct.	29.1b	32.6b	26.5b	32.3b	33.1b
Sum			74.5B	89.2A	71.0B	85.4A	81.1A

†Small case letters compare means between two sampling/harvest dates within same treatment and same location.
§Uppercase letter compare sums among treatments averaged across sampling/harvest dates within a same location.
For treatments, 0=0 kg K_2O ha^{-1} applied; 168=168 kg K_2O ha^{-1} applied at seeding, 168S=split-application, 84 kg K_2O ha^{-1} applied at first cut, 84 kg K_2O ha^{-1} applied in mid-Sept; 336=336 kg K_2O ha^{-1} applied at seeding; 336S= split-application, 168 kg K_2O ha^{-1} applied at first cut, 168 kg K_2O ha^{-1} applied in mid-Sept.

Table 12. Soil K unaccounted for, calculated as the difference between K in the soil in the spring plus the additional K in the fertilizer and the soil K in the fall plus K extracted by alfalfa in both cuts.

Location	168	168S	336	336S
Milnor	-----------------------------------kg of K ha^{-1}---			
RR Presteez	106	115	214	280
Stratica	79	108	229	280
L450 RR	79	104	212	277
Lisbon				
RR Presteez	142	151	314	347
Stratica	123	146	319	314
L450 RR	144	153	294	299
Mean treatment	113B	130B	264A	299A
Mean location				

Milnor	130B
Lisbon	180A
LSD loc x trt x var	

§Uppercase letter compare sums among treatments averaged across sampling/harvest dates and location.

Root protein and starch concentration

Root protein concentration was significant for K treatments averaged across sites, varieties, and harvest dates. The highest protein concentration was for the treatments without K fertilization and the non-stressed harvested alfalfa tended to have higher protein concentration than alfalfa stressed by harvesting on 18 September (Table 13). No interaction was observed between fall dormancy and K fertilization on root storage of protein on the seeding year of alfalfa.

The non-stressed alfalfa was harvested a month later (16 October). It makes sense that the protein concentration was higher in the non-stressed alfalfa, since the plants were able to keep translocating sucrose to the roots while the stressed alfalfa was harvested on 18 September, so translocation to the roots stopped at harvest. The higher protein concentration in plants without K fertilization is not the expected response. This study was done in soils with severe K deficiency so maybe adding K fertilizer promoted shoot growth in the fall, reducing root protein concentration.

Root starch concentration analysis has not been completed at the closing of this report. Since we did not see any effect of K fertilization on stand persistence, it is likely that in the seeding year K alone is not be able to overcome the stress caused by an untimely harvest in the fall.

Table 13. Root protein concentration with five K fertilizer treatments in stressed and non-stressed alfalfa plants.

Harvest date	0	168	168S	336	336S
	--------------------	--------------------	--------------------mg g^{-1}	--------------------	--------------------
18 September (stressed)	15.88ab	15.02bc	15.04b	15.12b	14.40c
16 October (non-stressed)	16.74a	15.57bc	16.28b	15.31b	15.87abc
Mean K treatment	16.31A	15.30B	15.66	15.22B	15.14B

Different small case letters indicate significant differences between the interaction of harvest date and K fertilizer treatment. Uppercase letters indicate differences between K fertilizer treatments averaged across harvest dates.

Conclusions

Higher fertilization rates of K_2O at seeding or split-application were not correlated with higher forage yield of alfalfa. Increase of K rates did result in a decrease in nutritive value, due to an increase in ash and decrease in TDN concentration. Alfalfa stand counts decreased from fall to spring in all treatments Alfalfa harvested on 18 September (stress treatment) had a stand reduction between 47.1 and 51.4%. Alfalfa harvested on 16 October (non-stress treatment) had lower stand reduction than the stressed treatment ranging between 16.5 and 26.9%. The significant differences in soil available K between locations supports that the higher smectite-to-illite clay ratios soil may have lower availability of K to plants. It is likely that part of the K_2O applied with the fertilizer was removed by the clays from the soil solution, making it unavailable.

The site with <3.5 smectite-to-illite ratio had an increase in soil K across all applications of K_2O, while the > 3.5 soil had no increase in soil K between sampling dates at any of the treatments. Split-application of the highest K rate resulted in the highest soil K value in the fall in the low (<3.5) smectite-to-illite soil. For the high smectite-to-illite soil, split-applications did not result in the highest available K in the fall. Regardless of treatment, soil K level in the fall did not increase above the recommended soil test level of 150 ppm needed in low smectite-to-illite ratio soils. Summing soil available K in the fall and K applied as fertilizer, the K accumulated in the plant explains less than 25% and 54% of the K available at the highest fertilization rate at the <3.5-clay ratio soil and >3.5-clay ratio soil, respectively. Protein concentration was lower in roots harvested on 18 September (stressed). K fertilizer rates tended to decrease root protein concentration.

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